DEVELOPMENT, CHARACTERIZATION, PROCESSING AND TESTING OF QUANTUM DOTS FOR IMAGING IN VISIBLE AND NEAR INFRARED RANGE

SYNOPSIS

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ASHA KUMARI

Enrollment Number: 146955

Under the supervision of

Dr. Ragini Raj Singh

DEPARTMENT OF PHYSICS AND MATERIALS SCIENCE

JAYPEE UNIVERSITY OF INFORMATION TECHNOLOGY
WAKNAGHAT, DISTRICT SOLAN, H.P., INDIA

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1. Introduction

Quantum dots (QDs) are a kind of nanocrystals attracting a greater deal of attention due to their confinement induced properties. In QDs electrons and holes are confined three dimensionally within Bohr exciton radius. The size of QDs is on nanometer scale and because of this small size QDs posses discrete electronic energy that give rise to unique optical properties [1]. Main defining characteristic of QDs such as size, optoelectronic properties as compared to bulk semiconductor material result in wide spectrum of applications in biological systems especially when compared with organic dyes. Such materials have advantages over traditional organic dyes such as these QDs have narrow and tunable emission spectra, high quantum yield and photostability [2]. Due to these fascinating properties of QDs they are considered as potential candidate for labeling, tracking and luminescent probes in biology [3, 4]. Visible II-VI group QDs like CdSe/ZnS QDs have been explored in multipurpose photonic applications like optical fibre amplifiers, solar cells, LED because of their brilliant and exceptional emission profiles. These visible QDs have also been utilized as optical temperature probes as well as in the fields of medicine and biology. In deep tissue imaging scattering and light absorption properties of tissues like proteins, hemoglobin and water reduces the fluorescence signal. This hampers the use of visible QDs in biological field. But NIR emitting QDs provides a clear window for animal tissues. Near infrared (NIR) light having wavelength longer than 650 nm and really escalate the ability of fluorescence in biomedical applications [5]. Based on all the discussed facts we have synthesized CdSe QDs, their core-shell and encapsulated structures (visible range) and CdTe QDs and their encapsulated structures (NIR range) for bioimaging applications.

Generally two major approaches could be used to synthesize QDs. One approach is organometallic route and the one is aqueous route [6, 7, and 8]. QDs synthesized by organometallic route are hydrophobic in nature and cannot be used directly for biological applications. Thus to use these QDs for biological application they require post treatment with some hydrophilic ligands [9]. In addition to this QDs should have less toxicity to use them for biological application. For this purpose we have synthesized hydrophilic visible and NIR QDs using aqueous route. After synthesis of QDs we have studied their structural, elemental and optical properties. On the basis of optical properties we found that these QDs posses poor optical properties thus to improve optical properties along with stability we have encapsulated these

QDs by polymers and silicates. After improvement in fluorescence property one major factor remained to study is cytotoxity of QDs. So we tested the toxicity of these QDs towards HEK-293 cell line.

2. Objective-1

Highlights of objective-1 involves the aqueous synthesis of poly CdSe and strongly confined CdSe QDs referred as CdSe1, CdSe2, CdSe4 and CdSe6 which were capped by 2-ME. Capping by 2-ME induce defects and these defects further increased with increase in 2-ME concentration. We demonstrate the removal of trap state emission from emission spectrum of CdSe QDs with increased intensity of band edge luminescence emission on polymer encapsulation. Out of all these prepared samples CdSe1, CdSe2, CdSe4 and CdSe6 we encapsulated CdSe4 QDs by different materials and selected it for further studies because in CdSe4 QDs intensity of band edge emission is more as compared to trap emission but in case of CdSe6 the intensity of trap emission dominates the band edge emission as well as QD size distribution is more in CdSe6.

2.1 Experimental

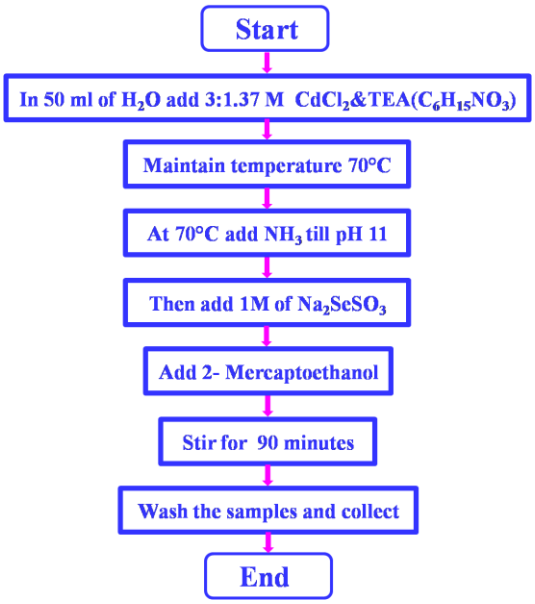
Start
In 50 ml of H_2O add 3:1.37 M $CdCl_2$&TEA($C_6H_{15}NO_3$)
Maintain temperature 70°C
At 70°C add NH_3 till pH 11
Then add 1M of Na_2SeSO_3
Add 2- Mercaptoethanol
Stir for 90 minutes
Wash the samples and collect
End

Figure 1: A process flow for aqueous synthesis of CdSe QDs

Figure 1 presents process flow for aqueous synthesis of CdSe QDs. 1 ml, 2 ml, 4 ml and 6 ml of 5 % 2-ME were used as capping agent to synthesize CdSe1, CdSe2, CdSe4 and CdSe6 respectively. After complete reaction the powder was filtered, centrifuged and dried at room temperature. Poly CdSe was synthesized using same procedure without the addition of 2-ME.

Encapsulated structures of CdSe4 QDs

QD surface stability is a major concern while synthesizing small CdSe QDs due to increased defects. To overcome this issue we have encapsulated CdSe4 QDs by silicate Tetraethylorthosilicate (TEOS) and polyethylene glycol (PEG) and poly vinyl alcohol (PVA). Encapsulated QDs were referred as CdSe4/TEOS, CdSe4/PVA and CdSe4/PEG QDs. 0.000125 moles of PVA was dissolved in distilled water to prepare CdSe4/PVA QDs. Reaction mixture was continuously put on a shaker for 48 hours. CdSe4/TEOS QDs and CdSe4/PEG QDs were synthesized by following the same methodology which was used for CdSe4/PVA QDs.

2.2 Results and Discussions

2.2.1 Structural analysis of Poly CdSe, CdSe1, CdSe2 and CdSe4 QDs

XRD spectra shown in figure 2 revealed that QDs include four main peaks at diffraction angles 2θ = 25.43, 42.12, 30.63 and 50°. These peaks are in a good conformity with cubic phase of CdSe QDs. Size calculated from XRD is tabulated in table 1.

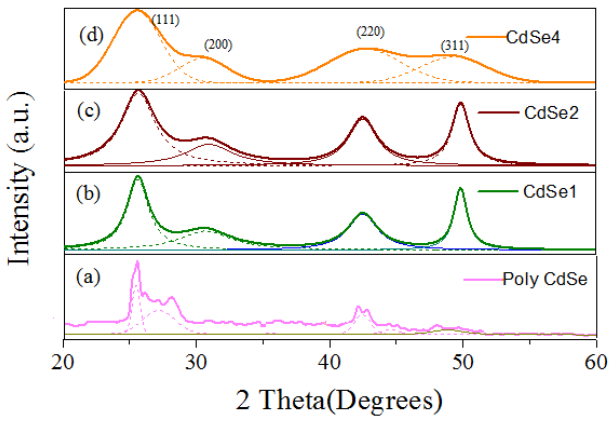

Figure 2: XRD spectra of synthesized (a) poly CdSe (b) CdSe1(c) CdSe2 and (d) CdSe4

Table 1: Structural parameters of poly CdSe, CdSe1, CdSe2, CdSe4 QDs, CdSe4/TEOS, CdSe4/PVA and CdSe4/ PEG

Sample Name	Size(XRD) (nm)	Size(TEM) (nm)	Surface/ Volume Ratio (nm^{-1})
CdSe4	1.8	1.8	3.15
CdSe2	2.5	2.6	2.4
CdSe1	3.7	-	1.62
Poly CdSe	22	-	-
CdSe4/TEOS	-	2.0	-
CdSe4/PVA	-	1.8	-
CdSe4/PEG	-	1.9	-

2.2.2 Transmission Electron Microscopy

TEM analysis confirms the spherical shape of prepared CdSe2, CdSe4, CdSe4/TEOS, CdSe4/PVA, and CdSe4/PEG. Particle size obtained from TEM is presented in Table 1.

2.2.3 Energy-dispersive X-ray Spectra

EDX spectra of QDs and their encapsulated structures were recorded to corroboration of elemental composition and encapsulation of QDs. Atomic percentage for all CdSe QDs and their encapsulated structures was confirmed from EDX. Obtained values of atomic percentage for all CdSe QDs are mentioned in table 2. Occurrence of sulphur in EDX spectra of CdSe1, CdSe2 and CdSe4 is due to 2-ME and atomic percentage of sulphur in these QDs is in increasing order and tabulated in Table 2. Attained stochiometric ratios for Cd: Se with different concentration of 2-ME is mentioned in table 3. As we can see in table 3 that ratio of Cd: Se is 1:0.63 in CdSe4 this ratio is not in good stochiometry as compared to ratio in case of CdSe1 and CdSe2 as tabulated in table 3. From table 3 it is also clear that sulphur content also increased on high concentration of 2-ME as it is 0.83 in CdSe4. While in case of polymer encapsulated QDs there is no peak of sulphur. This also confirms that there is no hole scavenging sulfide ions in CdSe4/PEG QDs, CdSe4/PVA and CdSe4/TEOS.

Table 2: Elemental Stochiometric ratio of CdSe1, CdSe2, CdSe4 CdSe4/PEG QDs, CdSe4/PVA QDs and CdSe4/TEOS QDs

ATOMIC PERCENTAGE (%)						
Elements	**CdSe1**	**CdSe2**	**CdSe4**	**CdSe4 /PEG**	**CdSe4 /PVA**	**CdSe4 /TEOS**
Cd	5.73	5.96	6.04	1.14	1.49	1.32
Se	5.28	4.80	3.87	1.15	1.07	0.63
O	4.11	2.26	1.26	3.58	-	3.92
S/Si	Nil	2.18/Nil	4.99/Nil	Nil	Nil	Nil/0.81 silica

Table 3: Elemental Stochiometric ratio of Cd: Se, Cd: S and Se: S in CdSe1, CdSe2, CdSe4, CdSe4/PEG QDs CdSe4/PVA QDs and CdSe4/TEOS QDs

Sample	**Conc. of 2-ME (in 50 ml solvent)**	**Ratio of Cd:Se**	**Ratio of Cd:S**	**Ratio of Se:S**
CdSe 1	1:50	1:0.98	1:00	1:00
CdSe2	2:50	1:0.80	1:0.36	1:0.45
CdSe 4	4:50	1:0.63	1:0.83	0.76:1
CdSe4/ PEG	PEG capped	1:1	1:00	1:00
CdSe4/ PVA	PVA capped	1:0.72	1:00	1:00
CdSe4/ TEOS	TEOS capped	1:0.47	1:00	1:00

2.2.4 Optical studies

Photoluminescence spectroscopy

Attained PL results show brilliant capping efficiency of 2-ME. However as we increase concentration of 2-ME this causes major variation to the emission spectra of QDs. The emission spectra of as prepared CdSe1 (low 2-ME 1ml), CdSe2, CdSe4 and CdSe6 (high 2-ME 6 ml) are presented in figure 3 (a). CdSe4 having high concentration of 2-ME show two distinctive

features in spectra (1) band edge emission (2) trap emission. Emission peaks with their position and intensity along with ratio of trap emission with respect to band edge emission for comparison for all samples are tabulated in table 4. Defect emission ratio was found high in case of CdSe4 because this contains high 2-ME as compare to CdSe1, CdSe2. Surface defects on QDs surface mainly dangling bonds originate traps and these traps have strong hole acceptor quality. Owing to higher possibility of non-radiative relaxation process related with dangling bonds, quantum efficiency of particles emitting from surface states is not very high [9]. As thiols are well-known for their hole scavenging property which cause quenching of band edge emission and boost the tendency of deep trap emission. In bare CdSe4 QDs trap emission arise due to selenium vacancies because in these QDs leakage chances of selenium are high because of weak bonding of Cd and Se. This study also confirms that surface interaction plays an important role in emission intensity of QDs. We encapsulated CdSe4 QD by polymer PEG, PVA and silicate TEOS. Comparative PL spectra of all CdSe4, CdSe4/TEOS, CdSe4/PVA and CdSe4/PEG presented in figure 3 (b) clarify that there is 10 fold increments in intensity along with complete exclusion of defects in case of silicate and polymer encapsulated structure. Results in Table 4 revealed that PL intensity is low in bare CdSe-QDs but on encapsulation intensity get enhanced.

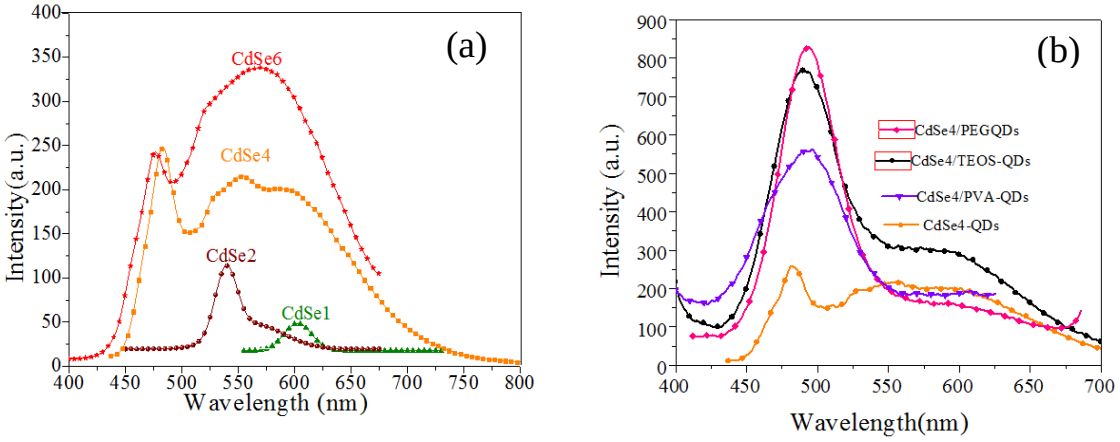

Figure 3: **(a)** Photoluminescence spectra of capped CdSe QDs with different concentration of 2-ME; where ▽-CdSe1; ●-CdSe2; □-CdSe4; ⁎-CdSe6. **(b)** Comparative PL spectra of CdSe4-QDs encapsulated with different polymers and silicates

Table 4: Summary of photoluminescence peak positions, corresponding FWHM and relative intensities for all CdSe QDs samples and their polymer encapsulated structures

Sr.No.	Name of Sample	PL Emission (nm)	FWHM (nm)	Intensity	Ratio of I_{eg}: Defect Intensity	Ratio comparison to the most intense CdSe4/PEG (in %)
1	**CdSe1**	600	24	49	1:0	-
2	**CdSe 2**	540	25	114	1:0.38	-
3	**CdSe 4**	482	31	259	1:0.86	31
4	**CdSe4/PEG**	492	47	828	1:0	100
5	**CdSe4/TEOS**	488	57	765	1:0.25	92
6	**CdSe4/PVA**	494	62	560	1:0	67

2.2.5 FTIR analysis

FTIR studies conclude that these QDs hold functional groups like -NH and -OH which formulate them to be hydrophilic and biocompatible. Fluorescent QDs prepared by aqueous route can be directly affixed to bio molecules due to their hydrophilic nature with great affinity. This is a boon of our technique, as the QDs prepared by high temperature organic synthesis routes are always possessing hydrophobic surfaces and need post treatment for biocompatibility and bioconjugation purposes. There are most commonly found functional groups on biomolecules are -OH, -CHO, -C=O, -NH and –SH. Most common band positions of CdSe4, CdSe4 QDs and their encapsulated structures have been assigned and presented in Table 5.

Table 5: Most common functional groups and band positions of CdSe4, CdSe4 encapsulated structures and their assignments

Assingments	Band Position
OH group	3346 cm^{-1}
Alkyne	2100 cm^{-1}
CH_2 rocking	Peaks ~ 1100 cm^{-1}
CH_2 bending	1462 cm^{-1}
NH bend,C=O stretching	1630 cm^{-1}
C-S Streching	672cm^{-1}
Silica	1089cm^{-1}

2.3 Conclusion

Present work concludes that increase in concentration of stabilizing agent i.e., 2-ME leads to formation of trap sites. Trap sites originated due to hole scavenging property of thiol group. Encapsulation of surface of small QD CdSe4 by polymer and silicates plays a significant role to

improve optical properties. The prepared QDs were characterized by XRD, TEM, EDX, absorbance spectroscopy, photoluminescence spectroscopy and FTIR. It has been found from photoluminescence spectroscopy that the QDs are of very small size with emission 480 nm. On rising 2-ME there is increase in surface to volume ratio. This causes increased number of unsatisfactory groups on surface of QDs and leads to defects. This study leads to confirmation that thiol content is responsible for trap emission beyond a particular concentration of 2-ME and this was also confirmed from EDX analysis. Other probable cause of trap emission was presence of dangling bond, high surface/volume ratio or moreover may be because of selenium vacancies. To overpower all these troubles we have encapsulated QDs by polymers and silica. PL studies noticeably revealed that QDs encapsulated with polymer show brilliant emission intensity with entire removal of trap emission. Considerably these polymer encapsulated QDs posses outstanding aqueous dispersibility enhanced trap free band edge emission intensity and improved fluorescence stability.

3. Objective-2

This objective describes the precursor based synthesis of zinc sulfide (ZnS) and CdSe/ZnS nanostructures and their structural, morphological, elemental, optical and functional analysis. Here we evaluated link between precursor reactivity, size of QDs and optical properties. It was found from optical studies that precursor used for synthesis of ZnS1 and ZnS2 affect the band edge emission and defect emission position in these QDs. Moreover, we have also studied the effect of ZnS shell on optical properties of core CdSe QDs.

3.1 Experimental

Synthesis of ZnS1 and ZnS2 QDs: Two different precursors of zinc, $ZnSO_4.7H_20$ and $ZnCl_2$ as zinc source were utilized to synthesize ZnS QDs ZnS1 and ZnS2 respectively. Different Zn precursors $ZnSO_4.7H_2O$ or $ZnCl_2$ and S precursor $(NH_2)_2CS$ were taken in 0.025:0.035 molar ratios. Aqueous chemical method was used to synthesize these ZnS QDs at 70^oC temperature.

CdSe/ZnS core/shell synthesis: Core-shell QDs were synthesized by seed growth method. The whole synthesis process for core-shell involves two-steps. First step involves synthesis of monodispersed core CdSe QDs even though these CdSe QDs were stabilized with 2-ME capping groups. Second step was synthesis of shell on these already prepared bare QDs. $ZnSO_4.7H_2O$

was used as precursor to synthesize CdSe/ZnS1 and $ZnCl_2$ was used to synthesize CdSe/ZnS2 core-shell QDs.

3.2 Results and Discussion

3.2.1 XRD analysis

XRD of core CdSe QDs show cubic phase. XRD pattern of ZnS1 (Figure 4 (a)), showed four peaks and these peaks are ascribed to pure cubic phase of ZnS1. It is clear from table 6 and figure 4 (b) that XRD of core/shell QDs contains prominent peaks situated at the centre of both CdSe and ZnS. Slight shift in 2θ positions in case of core-shell toward high 2 theta is clear confirmation of core-shell structure formation.

Table 6: Structural parameters of CdSe, ZnS1, ZnS2, CdSe/ZnS1 and CdSe/ZnS2 QDs

Sample Name	2θ Position	hkl	Size from XRD (nm)	Size from TEM (nm)
CdSe	25.47/30.39/42.58/49.11	111/200/220/311	1.8	1.9
ZnS1	29.18/33.08/48.73/58.13	111/200/220/311	2.8	3.1
ZnS2	28.74/**31.65**/**34.19**/**36.05(ZnO)** 47.48/56.55	111/100/002/101 220/311	2.5	2.5
CdSe/ZnS1	25.53/31.69/42.78/49.19	111/200/220/ 311	-	3.7
CdSe/ZnS2	25.49/31.69/34.57/36.19/42.61 47.74/56.69/62.76/67.89	111/100/002/101 220/220/311/013 112	-	3.5

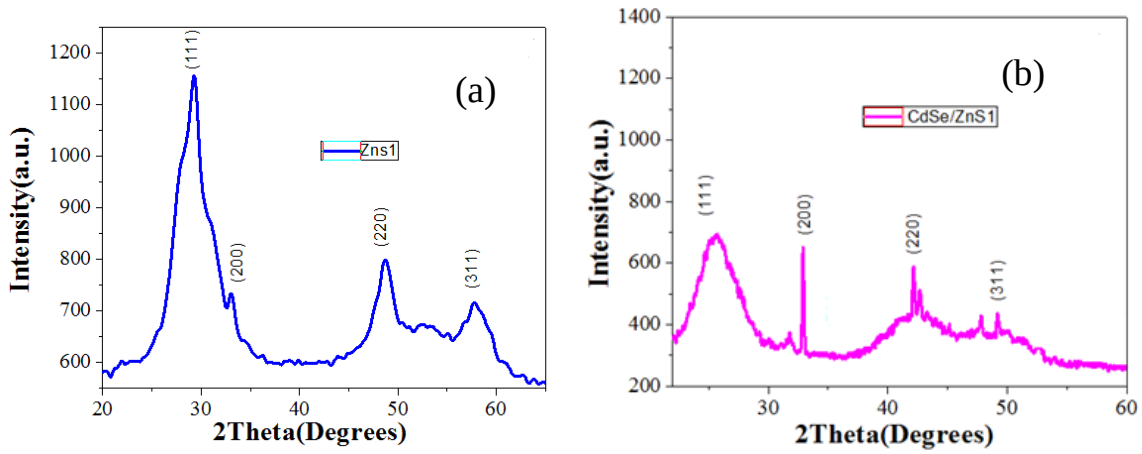

Figure 4: XRD spectra of synthesized (a) ZnS1 (b) CdSe/ZnS1

3.2.2 Transmission electron microscopy

TEM results of ZnS1, ZnS2 confirms spherical QDs with uniform size distribution. Particle size of ZnS1 was found larger as compared to ZnS2 due to variation in the precursor reactivity used

for synthesis of ZnS1 and ZnS2. Shell formation on CdSe core QDs by ZnS was also confirmed by TEM images. Obtained particle size for all samples has been mentioned in Table 6.

3.2.3 Energy-dispersive X-ray spectra

Synthesized QDs and their core/shell structures were analyzed by EDX for verification of elemental composition of samples. Stochiometric ratio of Zn: S and CdSe: ZnS for ZnS1, ZnS2, CdSe/ZnS1 and CdSe/ZnS2 QDs are tabulated in Table 7.

Table 7: Stochiometric ratio of Zn: S and CdSe: ZnS for ZnS1, ZnS2, CdSe/ZnS1 and CdSe/ZnS2 QDs

Sample	Ratio of Zn:S	Ratio of CdSe:ZnS
ZnS1	1:0.23	-
ZnS2	1:0.95	-
CdSe/ZnS1	-	1:0.35
CdSe/ZnS2	-	1:0.5

3.2.4 Optical studies

Photoluminescence and photoluminescence excitation (PLE) Studies

Excitation spectra for all QDs were recorded by setting emission on blue edge (band edge emission) of PL peak. In case of ZnS1 (Figure 5 (a)) excitation spectra of contain excitation peak at 333 nm which is associated to emission at 337, 427, 486 nm. Band edge emission for ZnS1 in PL was at 337 nm with defects states at 427 and 486 nm. ZnS2 shows excitation peak at 380 nm and this excitation peak correspond to band emission at 384 and defect emissions at 422 nm, 445 nm, 486 nm and 529 nm. In case of ZnS1 and ZnS2 emission peaks between 400-450 nm are associated with interstitial zinc and sulfur vacancies. Peaks at 485-490 nm were assigned to Zn-vacancies. Emission peaks at 525 nm is due to vacancy associated to recombination of electron from energy level of sulfur vacancy to hole on the energy level of Zn vacancy. Figure 5 (b)) shows excitation peak at 474 nm for CdSe/ZnS1 which correspond to emission at 487 nm along with defect emission at 531 nm respectively. Similarly, PLE spectra of CdSe/ZnS2 contain absorption peak at 472 nm this corresponds to PL emission at 484 nm with defect emission at position 532 nm. Presence of peak at 532 nm even after shell formation may be due to sulfur vacancies. It was confirmed from PL analysis that all QDs are fluorescent and fluorescent behavior of these QDs gets better on shell formation. Table 8 presents summary of PLE wavelength and emission wavelength for core CdSe, ZnS1, ZnS2, CdSe/ZnS1 and CdSe/ZnS2.

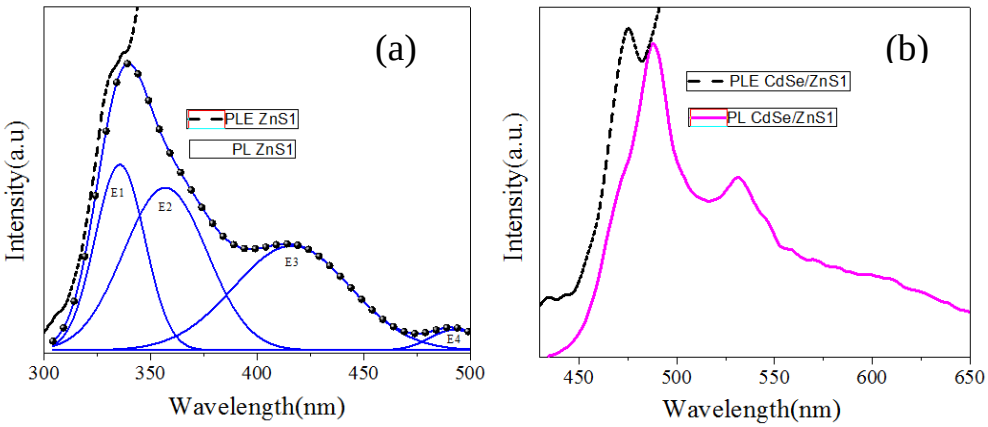

Figure 5: Photoluminescence spectra for **(a)** ZnS1; **(b)** CdSe4/ZnS1

Table 8: Summary of PLE wavelength and emission wavelength for core CdSe4, ZnS1, ZnS2, CdSe4/ZnS1 and CdSe4/ZnS2

Sample Name	PLE Wavelength(nm)	Emission wavelength(nm)
Core CdSe	476	481
ZnS1	333	337, 427, 486
ZnS2	380	385, 422, 445, 486, 529
CdSe4/ZnS1	474	487, 531
CdSe4/ZnS2	472	484, 532

3.2.5 FTIR analysis

FTIR spectra of ZnS1, ZnS2, CdSe/ZnS1 and CdSe/ZnS2 QDs confirm presence of NH and OH groups on QDs surfaces. Presence of these groups makes these QDs hydrophilic and biocompatible in nature. All FTIR band positions are presented in Table 9.

Table 9: FTIR most common functional groups and band position of ZnS1, ZnS2, CdSe4/ZnS1 and CdSe/ZnS2 their assignments.

Assignments	Band Position
OH group	~3200-3400 cm^{-1}
CH_2 bending	1462 cm^{-1}
CH_2 rocking	Peaks around 1100 cm^{-1}
NH bend	1630-1640 cm^{-1}

3.3 Conclusion

In this work we have found out the change in optical properties as an effect of ZnS precursor reactivity. Precursor reactivity also affects the size of QDs. From structural analysis of prepared QDs it was confirmed that the particle size of ZnS1 was greater than ZnS2. Optical analysis of QDs confirms that the precursors used to synthesize ZnS1 and ZnS2 influence the band edge and

defect emission position. In case of ZnS1 band edge emission was found at 337 nm along with few defects at 427 nm, 486 nm. As compared to ZnS1, in Zns2 band edge emission was at 384 nm along with defects at 422 nm, 445 nm, 486 nm and 529 nm. From all these studies we have successfully established the association between reactivity of precursor, optical properties and size of QDs.

4. Objective-3

Wurtzite CdTe NIR QDs have been synthesized using 3-MPA through aqueous route. These QDs provide emission in NIR optical diagnostic window. Encapsulation of these NIR QDs is procured to stabilize these QDs. NIR QDs play a more important role, particularly for detection in deep tissues and organs. Even though the quantum yield of NIR QDs is comparatively lower than that of visible QDs. But these NIR QDs after surface modification become more fluorescent than other fluorophores like dyes with emission in NIR. These NIR emitting QDs can be utilized for *in-vivo* applications because this is the range of spectrum where blood and tissue absorb minimally but still detectable by instrument. Thus NIR QDs emit at 700-1000 nm avoid or minimize problem of endogenous fluorescence of tissue.

4.1 Experimental

Procedure for synthesis of CdTe NIR QDs

Wet chemical route was used to synthesize CdTe NIR QDs. Figure 6 presents process flow for synthesis of CdTe NIR QDs. Poly CdTe was synthesized using same procedure but there was no addition of 3-MPA.

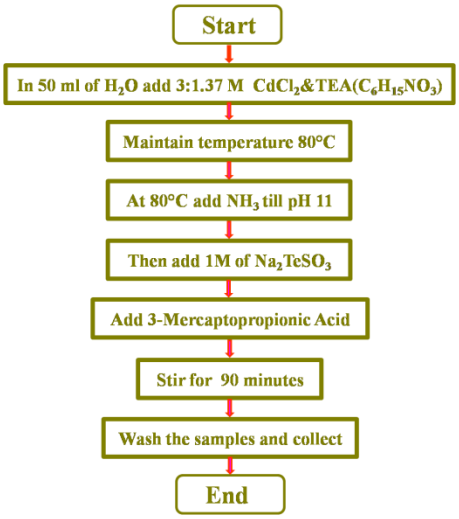
Start
In 50 ml of H_2O add 3:1.37 M $CdCl_2$&TEA($C_6H_{15}NO_3$)
Maintain temperature 80°C
At 80°C add NH_3 till pH 11
Then add 1M of Na_2TeSO_3
Add 3-Mercaptopropionic Acid
Stir for 90 minutes
Wash the samples and collect
End

Figure 6: A process flow for wet chemical synthesis of CdTe QDs

Encapsulated structures of CdTe QDs

Quantum dot surface stability and toxicity is a major concern for NIR CdTe QDs. To surmount this problem CdTe QDs were encapsulated by silicate Tetraethylorthosilicate (TEOS) and polymers polyethylene glycol (PEG) and poly vinyl alcohal (PVA). Encapsulated QDs were named as CdTe2/TEOS, CdTe2/PEG and CdTe2/PVA QD. 0.000125 moles of PEG was dissolved in distilled water to prepare CdTe/PEG QDs. Reaction mixture was continuously put on a shaker for 48 hours. CdTe2/TEOS QDs and CdTe2/PVAQDs were synthesized by following the same methodology which was used for CdTe2/PEG QDs.

4.2 Results and Discussion

4.2.1 Structural analysis of Poly CdTe, CdTe1, CdTe2 and CdTe 3 QDs

Figure 7 presents the XRD pattern of Poly CdTe, CdTe1, CdTe2, and CdTe3 QDs. As clear from the figure 7 peaks at 2θ = 23, 26, 34, 37, 42, 46 and 48° correspond to reflections due to (100), (101), (102), (110), (103), (200) and (202) planes assigned to hexagonal phase. Particle size of poly CdTe and CdTe QDs was calculated by most prominent peak 101 using Scherrer's formula. All the calculated structural parameters along with strain and crystallite size of CdTe1, CdTe2, CdTe3 QDs and poly CdTe are presented in the table 10.

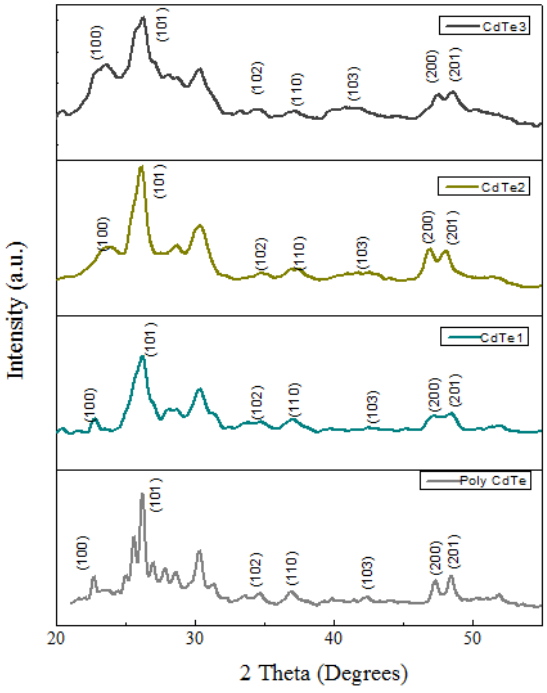

Figure 7: XRD pattern of Poly CdTe, CdTe1, CdTe2, and CdTe3 QDs

Table 10: Structural parameters of poly CdTe, CdTe1, CdTe2 and CdTe3 QDs

Sample Name	2θ (^{0})	FWHM (^{0})	Intensity	(hkl)	Size (nm)	Size from TEM (nm)
Poly CdTe	26.22	0.34	642	101	26	28
CdTe1	26.24	1.2	651	101	7	7.2
CdTe2	26.13	1.5	452	101	5	5.6
CdTe3	26.19	2.1	321	101	4.2	4.8

4.2.2 Transmission electron microscopy

Calculated particle size is tabulated in table 10. The obtained particle size was found less than Bohr exciton radius. Size acquired from TEM analysis was close to size as obtained from X-ray diffraction analysis.TEM analysis confirmed the non-agglomerated and spherically shaped QDs.

4.2.3 Energy-dispersive X-ray spectra

Obtained values for ratio of atomic percentage of Cd: Te from EDX analysis for all samples is tabulated in Table 11.

Table11: Value for ratio of atomic percentage of Cd: Te from EDX analysis

Sample	Ratio of Cd:Te	Ratio of Cd:Te:Si
Poly CdTe	1:0.79	-
CdTe1	1:0.76	-
CdTe2	1:0.63	-
CdTe3	1:0.55	-
CdTe2/TEOS	1:0.66	1:0.66:0.78
CdTe2/PVA	1:0.81	-
CdTe2/PEG	1:0.89	-

4.2.4 Optical studies

Photoluminescence and PLE spectra of NIR QDs were recorded by using 0.02 mg/ml of solution. Figure 8(a) shows effect of stabilizing agent 3-MPA on PL emission of CdTe1, CdTe2 and CdTe3 in the form of blue shift in emission peak on increasing the concentration of 3-MPA. The emission peak of CdTe3 contains high concentration of 3-MPA get blue shifted as compared to CdTe1. This proves superior capping efficacy of 3-MPA. All the emission peaks and PLE peaks for CdTe1, CdTe2 and CdTe3 are tabulated in Table 12. Out of all these CdTe1, CdTe2 and CdTe3 QDs we have selected CdTe2 for further analysis because CdTe2 shows emission in NIR range. Although CdTe1 shows emission in NIR range but its stability is less as compared to CdTe2 whereas CdTe3 QDs were emitting in visible range. To remove the defects from CdTe2

we have encapsulated CdTe2 by PEG, PVA and TEOS. Figure 8 (b) shows comparative spectra of CdTe2 and their polymer encapsulated structure CdTe2/PEG, CdTe2/PVA and CdTe2/TEOS and also show complete removal of defects from polymer and silicate encapsulated structures. Along with defect removal, intensity of polymer and silicate encapsulated QDs get improved. Emission peak positions with compared intensity for CdTe2 QDs, CdTe2/PEG, CdTe2/PVA and CdTe2/TEOS structures are demonstrated in Table 13. Results indicate that PL intensity is low in bare CdTe2 however on encapsulation intensity gets improved by two to four folds. This improvement in fluorescence intensity by encapsulation is because of smoothening of QDs surface.

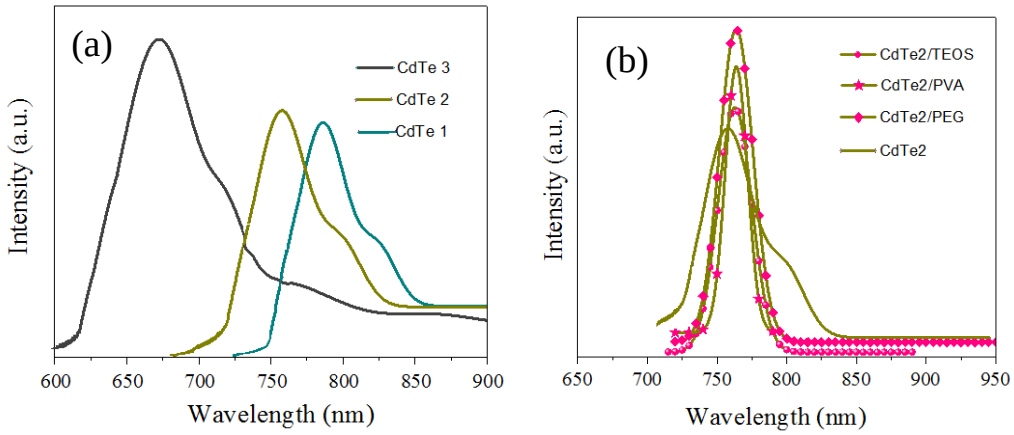

Figure 8: (a) Photoluminescence spectra of capped CdTe QDs with different concentration of 3-MPA **(b)** Comparative PL spectra of CdTe2-QDs encapsulated with different polymer and silicates

Table 12: Emission peaks and PLE peaks of CdTe1, CdTe2 and CdTe3

Sample Name	PLE Wavelength (nm)	Emission wavelength (nm)
CdTe1	778	786,825
CdTe2	744	757,799
CdTe3	657	672,720, 758,862

Table 13: Summary of emission positions, corresponding FWHM and relative %age intensity of CdTe2, CdTe2 /TEOS, CdTe2/PVA and CdTe2/PEG

Sample Name	Spectral position of PL emission	FWHM	%age intensity increase
CdTe2	757	40	1%
CdTe2/TEOS	762	28	10%
CdTe2/PVA	763	20	28%
CdTe2/PEG	764	29	45%

4.2.5 FTIR analysis

FTIR results of poly CdTe, CdTe1, CdTe2 and CdTe3 shows peaks at 3430 $cm^{-1,}$ 2840 cm^{-1}, 1400 cm^{-1}, 1066 cm^{-1} and 875 cm^{-1}. The presence of these peaks at particular wavelengths are due to -OH group, -CH stretching,-CH2 bending, C-O stretching and C-S stretching respectively. Peak at 1541 cm^{-1} is characteristic peak of nitro group. Similarly, in FTIR spectra for CdTe2/PVA, CdTe2/PEG and CdTe2/TEOS presence of two intense peaks one at 3320 cm^{-1} was assigned to OH and at 1640 cm^{-1} be a sign of -NH group. FTIR results of these NIR QDs confirm hydrophilic nature of surface modified QDs.

4.3 Conclusion

This work concludes that capping of NIR CdTe QDs by 3-MPA controls the particle size. This decrease in particle size leads to shift in PL emission peak position. TEM, XRD, EDX and PL analysis confirms structural, elemental, optical properties of CdTe. From PL analysis of CdTe it was confirmed that CdTe1, CdTe2 shows emission in NIR range. Owing to emission of these QDs in NIR range these can be utilized for biological applications in future.

5. Objective-4

This work focuses on cytotoxicity and antimicrobial studies of CdSe based and CdTe based quantum dot structures. Cytotoxicity testing of CdSe and CdTe based material was carried out toward HEK-293 cells. Cytotoxic effect of these materials was evaluated by using MTT assay. After confirmation of toxic effects by MTT assay cellular apoptosis caused by QDs was further analyzed for better understanding of the toxicity by DAPI/AO/PI fluorescent staining on HEK-293 cells. Hereafter this chapter also includes study of antimicrobial effect of CdSe4, ZnS1, ZnS2, CdSe4/ZnS1 and CdSe4/ZnS2 QDs by Kirby Bauer's disk diffusion method against potential gram negative pathogens; *Escherichia coli* ATCC 25922 and *Acinetobacter baumannii* ATCC 19606. Antimicrobial study for poly CdTe, CdTe2 and polymer, silicates encapsulated structures were performed against gram negative and gram positive pathogens.

5.1 Experimental

5.1.1 Procedure for cell cytotoxicity of CdSe, CdTe based nanostructures

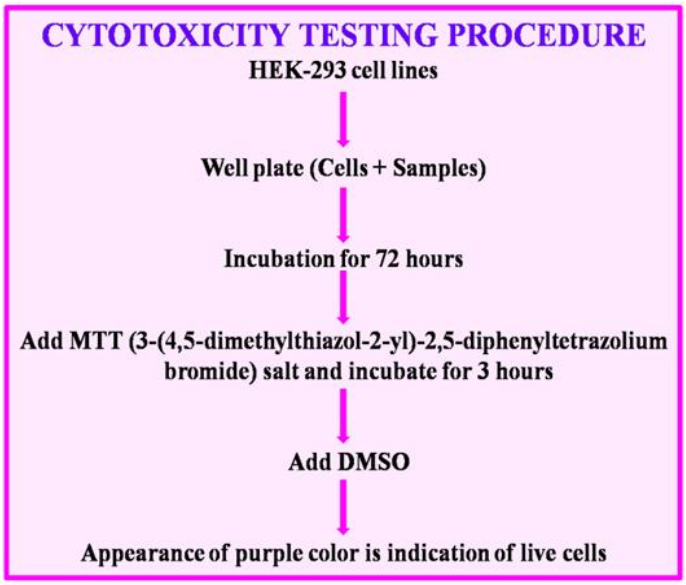
CYTOTOXICITY TESTING PROCEDURE
HEK-293 cell lines
Well plate (Cells + Samples)
Incubation for 72 hours
Add MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) salt and incubate for 3 hours
Add DMSO
Appearance of purple color is indication of live cells

Figure 9: Procedure for cell cytotoxicity of CdSe, CdTe based nanostructures

5.1.2 Antimicrobial susceptibility testing of CdSe based nanostructures

Kirby Bauer's disk diffusion method was used to screen antibacterial activity of CdSe based QDs and their core-shell structures against potential gram negative pathogens; *Escherichia coli* ATCC 25922 and *Acinetobacter baumannii* ATCC 19606. Standard guidelines of CLSI (Clinical laboratory standard institute) and ICMR (Indian council of medical research) were used to perform these studies. Bacterial cultures were inoculated in Luria broth and were incubated at 37°C for overnight. 0.5 O.D. McFarland standards bacterial culture was swabbed onto Muller Hinton agar plate and permitted to dry for 15 minutes. Disks of control antibiotics and synthesized compounds were positioned onto agar plates. Plates were transferred in to an incubator and maintained at 37°C for 24 hours and zones of inhibition around disk were read according to ICMR and CLSI guidelines.

5.1.3 Antimicrobial susceptibility testing of CdTe based nanostructures

Antimicrobial testing of CdTe based nanostructures was studied by Kirby Bauer's disk diffusion method against gram –ve and gram +ve bacteria. Bacterial cultures were inoculated in Luria broth and were incubated at 37°C for overnight. 0.5 O.D. McFarland standards bacterial culture was swabbed onto Muller Hinton agar plate and permitted to dry for 15 minutes. Disks of control antibiotics and synthesized compounds were positioned onto agar plates. Plates were transferred in to an incubator and maintained at 37°C for 24 hours and zones of inhibition around disk were read according to ICMR and CLSI guideline.

5.2 Results and Discussions

5.2.1 Cytotoxicity studies of CdSe based nanostructures

Cytotoxicity of QDs toward HEK-293 cells was evaluated by using MTT assay. The cells were exposed for 12 h, 24 h and 72 h with increasing concentrations of QDs (0 to 1000 µg/ml). Cell viability was systematically deliberated to explain the toxicity. 24 h of incubation period shows prominent reduction in cell viability for considerable conclusion. It was clear from figure 10 that QDs show signs of concentration reliant reduction in cell viability. Out of all the prepared QDs, Poly-CdSe was most toxic with IC_{50} values 440.84 µg/ml at 12 h and 251.19µg/ml at 24 h, similarly CdSe-QDs correspond to IC_{50} values of 529.71 µg/ml and 397.94 µg/ml for 12 h and 24 h respectively. Cytotoxic outcome in case of CdSe/ZnS1 QDs was time and dose dependent but, CdSe/ZnS1 demonstrate less anti-proliferative activity in comparison to Poly-CdSe with increase in IC_{50} value by 3.2 fold, 4.2 fold and 2.3 fold for 12 h, 24 h and 72 h respectively (Table 14).

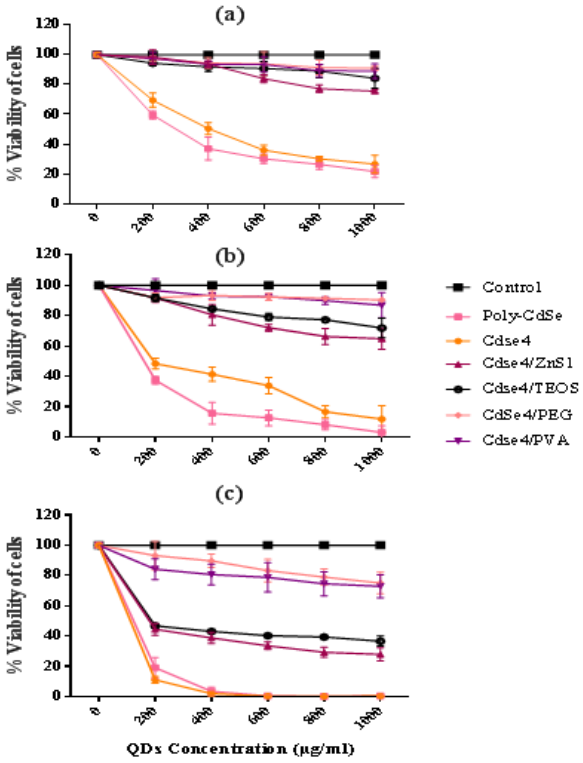

Figure 10: Viability of HEK-293 cells after treatment with synthesized QDs at different concentration for 12 hours (a) 24 hours (b) and 72 hours (c) Percentage cell viability was calculated with respect to viability of the un-treated (control) cells and which was taken as 100 %. The results are mean ± SD from three indented experiments

In the same way, IC_{50} values for CdSe/TEOS also rise by 3.1 fold, 5.5 fold and 2.3 fold for 12 h, 24 hand 72 h respectively (Table 14). CdSe/PVA QDs illustrate 8.8 fold, 15.1 fold and 13.1 fold rise in IC_{50} for 12 h, 24 h and 72 h in comparison to Poly-CdSe. Fascinatingly, CdSe/PEG QDs illustrate 13.7 folds, 23.3 folds and 14.2 folds rise in IC_{50} for 12 h, 24 h and 72 h (Table 14). Biocompatibility of QDs was attained in subsequent order, CdSe 4/PEG QDs > CdSe 4/ PVA QDs> CdSe 4/TEOS QDs > CdSe 4/ZnS1 QDs >CdSe 4 QDs > Poly CdSe.

Table 14: Corresponding IC_{50} values and fold change in IC_{50} values of synthesized QDs on HEK-293 cells proliferation at 12 h, 24 h and 72 h with respect to Poly-CdSe

	12 Hours		24 Hours		72 Hours	
	IC_{50} (µg/ml)	Fold Change	IC_{50} (µg/ml)	Fold Change	IC_{50} (µg/ml)	Fold Change
Poly CdSe	440.84	-	251.19	-	131.19	-
CdSe4	529.71	0.2	397.94	0.5	92.45	-0.30
CdSe4/ZnS1QD	1862.84	3.2	1287.85	4.2	427.10	2.3
CdSe4/TEOS QD	1764.12	3.1	1621.12	5.5	521.99	2.3
CdSe4/PVA QD	4352.55	8.8	4020.96	15.1	1844.20	13.1
CdSe4/PEG QD	6518.24	13.7	6124.24	23.3	1989.48	14.2

5.2.2 Cytotoxicity studies of CdTe based nanostructures

Reduction in cell viability was enough prominent at 24 h of incubation for note worthy interpretation (This conclusion we have made on the basis of results obtained from CdSe cytotoxicity studies). As depicted in Figure 11, QDs showed the concentration dependent decline in the cells viability. Poly CdTe exhibited most toxic effect on the cells with IC_{50} of 6.95 µg/ml, whereas CdTe2 represented IC_{50} 8.27 µg/ml. After successful encapsulation of QDs with PVA, PEG and TEOS we witnessed decline in the toxic effects, where, CdTe2/PEG was found to be least toxic (IC_{50} values =548.08 µg/ml). CdTe2/PVA and CdTe2/TEOS exhibited IC_{50} values of 306.55 µg/ml and 129.30 µg/ml respectively (Table 15).

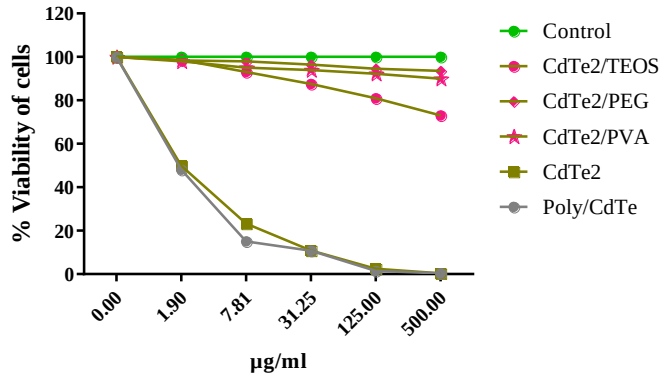

Figure 11: Viability of HEK-293 cells after treatment with synthesized QDs at different concentration for 24hrs

Table 15: Table showing individual IC_{50} value for CdTe QDs

Sr.no	Sample	IC50 Value
1	Poly CdTe	6.95
2	CdTe 2	8.27
3	CdTe2/ PVA	306.55
4	CdTe2/PEG	548.08
5	CdTe2/TEOS	129.30

5.2.3 Antimicrobial studies

Antimicrobial studies of CdSe based nanostructures

Antimicrobial susceptibility testing was done by Kirby Bauer's disk diffusion method demonstrated that two compounds A and F having effective antibacterial activity against gram negative pathogens (Table16).QDs whose antimicrobial study was carried out were referred as compound A-CdSe4, Compound B-ZnS1, Compound C-ZnS2, Compound D-CdSe4/ZnS1, compound E-CdSe4/ZnS2 and compound F-poly CdSe respectively. *E. coli* strains cause intestinal as well as extra-intestinal infectivity [37]. Another pathogen which we have used in our study was *Acinetobacter baumannii* which is regarded as foremost cause of nosocomial pathogen, these results in wide range of blood, respiratory, skin and soft tissue infections. In recent times, *A. baumannii* has been listed as the chief multidrug resistant bacteria among hospital settings by World Health Organization.

Table 16: Antimicrobial susceptibility tests of compounds against gram negative pathogens

Concentration of compounds in µg/ml	**Zone of lysis in mm**											
	CdSe4		**ZnS1**		**ZnS2**		**CdSe4/ZnS1**		**CdSe4/ZnS2**		**Poly CdSe**	
	E. coli	***A. baumannii***	***E. coli***	***A. baumannii***	***E. coli***	***A. baumannii***	***E. coli***	***A. baumannii***	***E. coli***	***A. baumannii***	***E. coli***	***A. baumannii***
1024	29	-	-	-	-	-	-	-	-	-	20	25
512	16	-	-	-	-	-	-	-	-	-	17	23
256	13	-	-	-	-	-	-	-	-	-	15	21
128	12	-	-	-	-	-	-	-	-	-	12	19
64	10	-	-	-	-	-	-	-	-	-	-	17
32	-	-	-	-	-	-	-	-	-	-	-	16

From Table 16 it is clear that CdSe4 (Figure 12) and poly CdSe are showing good antimicrobial activities. Elemental composition of CdSe4 QDs and Poly CdSe was very toxic due to heavy metals which were confirmed from EDX. Because of this toxic composition of CdSe4 QDs they show good antimicrobial activity. As compared to CdSe4 and poly CdSe, ZnS1 and ZnS2 QDs do not show any sign of antimicrobial activity means no formation of zone of inhibition in ZnS1 and ZnS2 (Table16). Bactericidal performance of CdSe4 QDs has also been accredited to their small size in contrast to ZnS1 and ZnS2. Smaller size of CdSe4 QDs has been confirmed from XRD and TEM analysis as compare to ZnS1 and ZnS2. Likewise CdSe/ZnS1 and CdSe/ZnS2 do not confirms any antimicrobial activity because of increase in size on shell

formation. This shell creation on core CdSe4 also stops leakage of heavy toxic metal and prevent photo oxidation of core material.

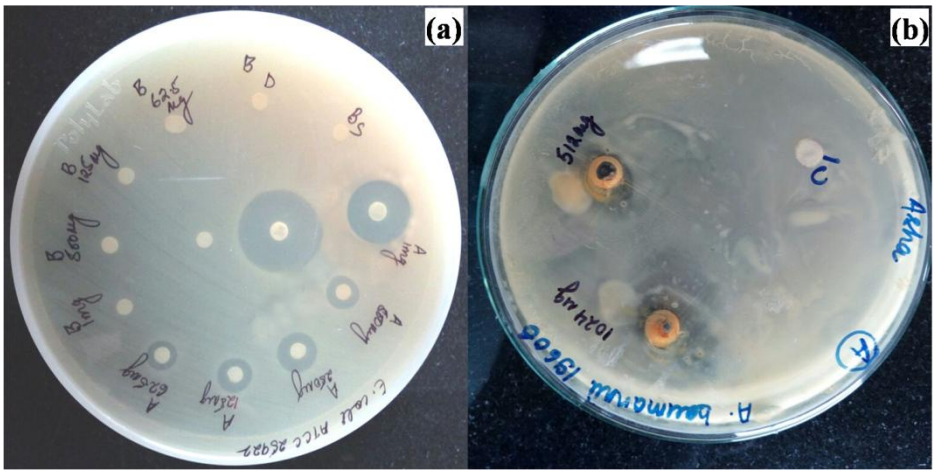

Figure12: Antimicrobial behavior of CdSe4 QDs

5.2.4 Antimicrobial studies of CdTe based nanostructures

Antimicrobial susceptibility testing was done by Kirby Bauer's disk diffusion methods for poly CdTe, CdTe2 QD, CdTe2/PVA, CdTe2/PEG and CdTe2/TEOS. From antimicrobial results it was confirmed that CdTe2 QDs and poly CdTe were showing good antimicrobial activity while no formation of zone of inhibition in case of CdTe2 encapsulated structures.

5.3 Conclusion

Cytotoxicity concern of CdSe based and CdTe based nanostructures was systematically studied and assessed. For CdSe based nanostructures poly CdSe and CdSe4 QDs were found highly toxic. Similar pattern was seen in case of CdTe where poly CdTe was most toxic as compared to other encapsulated structures. High toxicity of poly CdSe, CdTe was because of release of toxic ions. In comparison with bare QDs, encapsulated CdSe based QDs are nontoxic still at very high concentrations and long incubation time of 24 hours and even up to 72 hours of incubation. Slight difference was found in cell viability for 24 and 72 hours in case of CdSe QDs. Non toxic nature of encapsulated CdSe and CdTe QDs is probably due to occurrence of layer on these QDs. This encapsulation layer prevents degradation and release of toxic ions.

Antimicrobial behavior of all synthesized QDs was studied against *Escherichia coli* ATCC 25922 and *Acinetobacter baumannii* ATCC 19606. Poly CdSe and CdSe4 QDs were showing superior antimicrobial activity as of their toxic composition. As compare to CdSe4 QDs, ZnS1 and ZnS2 QDs do not illustrate any antimicrobial behavior. Bactericidal performance

of CdSe4 QDs has also been accredited to their small size in contrast to ZnS1 and ZnS2. CdSe/ZnS1 and CdSe/ZnS2 does not show any antimicrobial property. PL spectra clearly divulge that fluorescent property of these QDs get enhanced on shell formation. Similarly in case of CdTe based nanostructures CdTe poly and CdTe2 QDs were showing antimicrobial activity. Polymer encapsulated structures of CdTe2 were not showing any kind of antimicrobial behavior. So from these results it was confirmed that polymer encapsulated CdTe nanostructures were nontoxic in nature toward the microbes. These findings also support our cytotoxic results.

6. References

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9) Pellegrino T., Manna L., Kudera S., Liedl T., Koktysh D., Rogach AL., Keller S., Rädler J., Natile G, Parak WJ., " Hydrophobic nanocrystals coated with an amphiphilic polymer shell: a general route to water soluble nanocrystals" Nano letters, Vol14;4(4), pp.703-7, Apr 2004.

7. PUBLICATIONS IN INTERNATIONAL REFEREED JOURNALS:

1. **Asha Kumari**, Ragini Raj Singh (2017). Encapsulation of Highly Confined CdSe Quantum Dots for Defect Free Luminescence and Improved Stability. *Physica E: Low-dimensional Systems and Nanostructures, 89*, 77-85. (Scopus/SCI, I.F. 2.2)

2. **Asha Kumari**, Arun Sharma, Udabanu Malairaman and Ragini Raj Singh (2018). Proficient surface modification of CdSe quantum dots for highly luminescent and biocompatible probes for bioimaging: A comparative experimental investigation. *Journal of Luminescence*, 199, 174-182. (Scopus/SCI, I.F. 2.6)

3. Hitanshu Kumar, **Asha Kumari** and Ragini Raj Singh (2017). Tunable narrow emission in ZnS/CdS/ZnS quantum well structures prepared by aqueous route. *Optical Materials, 69*, 23-29.(Scopus/SCI, I.F. 2.2)

4. **Asha Kumari**, Ragini Raj Singh (2017). Cytotoxicity testing of bare CdSe quantum dots and their encapsulated structures.AIP conference Proceedings, 1860, 020003(Scopus).

8. RESEARCH PAPERS COMMUNICATED IN INTERNATIONAL REFEREED JOURNALS

1. Asha kumara, Nutan thakur and Ragini Raj singhEvaluation of structural, morphological, elemental, optical, functional and antimicrobial properties of ZnS (precursor based), CdSe and CdSe/ZnS quantum dots. *Material Science and Engineering: C.*

9. CONFERENCES/WORSHOPS ATTENDED:

1. "Optical properties of CdSe Encapsulated with Polymer structures" Asha Kumari and Ragini Raj Singh, National Conference on **Multifunctional Advanced Materials (11-13 May 2016),** Shoolini University, H.P.

2. "Optical Properties of CdSe/ZnS core/shell Structures**"** Asha Kumari and Ragini Raj Singh, National Conference on **Smart Materials: Advances in Research and Techniques (SMART-2015)** (26-27 Nov, 2015), Shoolini University, H.P.

3. Workshop on **"Advanced Nanomaterials: Applications in Disease Diagnoses and Imaging"** (May 29-June 3, 2016) Global initiative of Academic Networks (GIAN), Ministry of Human Resource Development Govt. of India.

4. Cytotoxicity testing of bare CdSe Quantum Dots and their Encapsulated Structure**"** Asha Kumari and Ragini Raj Singh, International conference on **Recent Advances in Fundamental and Applied Science** (25-26 November 2016) .

5. "Effect of Stabilizing Agent and Encapsulation on Optical Properties of CdSe Quantum dots ICMR sponsored National Conference on **"Advances in Basic & Applied Sciences (ABAS-2017)".**